EXPERT OPINION

Nanoemulsions as potential vehicles for transdermal and dermal delivery of hydrophobic compounds: an overview

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Introduction: In recent years, nanoemulsions have been investigated as potential drug delivery vehicles for transdermal and dermal delivery of many compounds especially hydrophobic compounds in order to avoid clinical adverse effects associated with oral delivery of the same compounds. Droplet size and surface properties of nanoemulsions play an important role in the biological behavior of the formulation.

Areas covered: In this review, current literature of transdermal and dermal delivery of hydrophobic compounds both *in vitro* as well as *in vivo* has been summarized and analyzed.

Expert opinion: Nanoemulsions have been formulated using a variety of pharmaceutically acceptable excipients. In many cases of dermal and transdermal nanoemulsions, the skin irritation or skin toxicity issues on human beings have not been considered which needs to be evaluated properly. In the last decade, much attention has been made in exploring new types of nanoemulsion-based drug delivery system for dermal and transdermal delivery of many hydrophobic compounds. This area of research would be very advantageous for formulation scientists in order to develop some nanoemulsion-based formulations for their commercial exploitation and clinical applications.

Keywords: dermal delivery, drug delivery vehicles, hydrophobic compounds, nanoemulsions, transdermal delivery

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1. Introduction

Dermal drug delivery is the topical application of drugs to the skin surface to treat local skin diseases [1], whereas transdermal drug delivery is transport of systemically absorbed drugs through the intact skin into systemic circulation to treat various chronic disorders such as hypertension, arthritis, diabetes, cancer, etc. Dermal and transdermal drug delivery system offer many advantages over oral drug delivery system such as avoidance of hepatic first-pass metabolism, reduction of side effects and enhancement in solubility/bioavailability of drugs, etc. [1,2]. Most new compounds discovered and many existing ones are poorly soluble in an aqueous medium. Poor solubility in physiological fluids as well as poor permeability through the gastrointestinal (GI) membrane limits *in vivo* absorption and thus bioavailability which is an obstacle to drug development [3,4]. Indeed, effective delivery of compounds is significantly influenced by the physicochemical properties such as dissolution rate, solubility, and partition coefficient. Many formulation strategies have been devised for improving the solubility and bioavailability of hydrophobic compounds. Conventional techniques, such as complexation with β-cyclodextrins (β-CDs) or caffeine [5,6], salt formation [7], conjugation to dendrimers [8]

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Article highlights.

- Conventional techniques, such as complexation with β-CDs or caffeine, salt formation, conjugation to dendrimers and use of co-solvents have been employed to solubilize hydrophobic compounds. However, universal techniques that can significantly enhance the solubilization as well as *in vivo* bioavailability of these compounds are still required.
- Recently, nanotechnology-based formulations such as NE, ME, SLN, NLC, drug nanocrystals, niosomes/proniosomes and liposomes have been successfully used for bioavailability enhancement of hydrophobic compounds.
- One of the most promising methods to enhance solubilization and bioavailability of these poorly soluble drugs is formulation of NE.
- NEs have higher solubilization capacity than other dispersions and their thermodynamic stability offers many advantages over unstable dispersions, such as emulsions and suspensions, etc. and they can be manufactured with little energy input and have long shelf life.
- The application of transdermal delivery to a wider range of drugs is limited due to the significant barrier to penetrate the skin. This significant effort has been devoted to developing strategies to overcome the impermeability of intact human skin. One of these strategies is the use of NE technique in the transdermal drug delivery.

This box summarizes key points contained in the article.

and use of co-solvents [9,10] have been employed to solubilize these compounds. However, universal techniques that can significantly enhance the solubilization as well as *in vivo* bioavailability of these compounds are still required.

Recently, nanotechnology-based formulations such as nanoemulsions (NE) [11], microemulsions (ME) [12], solid lipid nanoparticles (SLN) [13], nanostructured lipid carriers (NLC) [14], drug nanocrystals [15], niosomes/proniosomes [16] and liposomes [17] have been successfully employed for bioavailability enhancement of hydrophobic compounds. As an evidence of this success, several nanotechnology-based formulations have been approved by the United States Food and Drug Administration (FDA) for the treatment of many diseases. One of the most promising methods to enhance solubilization and bioavailability of these poorly soluble compounds is the formulation of NEs. NEs prepared by low-energy emulsification (LEE) techniques (e.g., spontaneous emulsification or phase inversion temperature) are thermodynamically stable dispersions of oil and water stabilized by an interfacial film of surfactants usually in combination with a cosurfactants, where the droplet size is less than 100 nm [11]. NEs offer many advantages over emulsions, suspensions, liposomes, niosomes, nanoparticles, etc. These advantages include higher solubilization capacity, small droplet size, physical stability, lower viscosity, ease of preparation, bioavailability enhancement, etc. They can be manufactured with little energy input and have a long shelf life [18-20].

Droplet size and surface properties of NEs play an important role in the biological behavior of the formulation [18]. Small droplet sizes lead to transparent products such that their appearance is not altered by the addition of an oil phase. The attraction of oil-in-water (o/w) NE lies in their ability to hold hydrophobic compounds in their oil phase and thereby enhancing their solubilization as well as *in vivo* bioavailability [19].

In comparison to other nanotechnology-based products, there are fewer NE-based formulations approved or under clinical investigations for therapeutic use (**Table 1**). Most of the NEs available in the international market are recommended either for topical application or for oral administration. None of the marketed NEs have been approved for transdermal delivery of a hydrophobic compound. Transdermal drug delivery system (TDDS) of hydrophobic compounds through the skin to the systemic circulation provides a convenient route of administration for a variety of clinical indications. The application of transdermal delivery of a wider range of drugs is limited due to the significant barrier of the penetration of the skin [11,16]. Thus, substantial efforts have been devoted for developing strategies to overcome the impermeability of intact human skin. One of these strategies is the use of NEs for transdermal delivery [11]. Therefore, the aim of this article is to analyze and summarize the current literature associated with transdermal and dermal delivery of hydrophobic compounds using NE as vehicles both *in vitro* as well as *in vivo*. Successful and flawed strategies are considered to elucidate the best approaches to dermal and transdermal drug delivery using NE.

2. Classification of nanovehicles

The different nanovehicles that have been used extensively for drug delivery and targeting can be classified as lipid-based nanovehicles, polymeric nanovehicles, metallic nanovehicles, biological nanovehicles, etc. Lipid-based and polymeric nanovehicles have been used extensively for dermal and transdermal drug delivery:

2.1 Lipid based nanovehicles

2.1.1 Microemulsions

MEs are thermodynamically stable transparent isotropic colloidal dispersions of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecule having droplet size 20 – 140 nm [12,19]. It is very difficult to distinguish between NEs and MEs because their composition and appearance is nearly same. Nevertheless, MEs can be distinguished from NEs based on following properties [21-23]:

- ME forms spontaneously and typically required higher surfactant to cosurfactant ratio than a NE.
- Ultra low value of interfacial tension is required for formulation of ME than a NE.
- The shape of droplets may be spherical or nonspherical in case of ME as compared to spherical shape of NE.
- MEs have a longer shelf life than NEs.

Table 1. Commercial nanotechnology-based formulations of hydrophobic compounds that have been approved for clinical use or under clinical trials.

Product (Drug)	Formulation type	Indication	Company (Status)	ROA
Neoral® (Cyclosporin)	Liquid filled soft gelatin capsule	Immunosuppresion	Novartis Pharmaceuticals (Approved)	Oral
Norvir® (Ritonavir)	Semi-solid filled hard gelatin capsules	Antiviral	Abbott Laboratories (Approved)	Oral
Fortovase® (Saquinavir)	Liquid filled soft gelatin capsule	Antiviral	Roche Pharmaceuticals (Approved)	Oral
Agenarase® (Amprenavir)	Liquid filled soft gelatin capsule	Antiviral	GlaxoWellcome (Approved)	Oral
Estrasorb® (Estradiol)	Topical emulsion	Vasomotor symptoms associated with menopause	Novavax (Approved)	Topical
Flexogan® (Methy salicylate)	Topical emulsion	Analgesic	AlphaR$_x$ (Approved)	Topical
Restatis® (Cyclosporin	Ophthalmic emulsion	Chronic dry eye disease	Allergan (Approved)	Opthalmic
Oxalginnanogel® (Diclofenac sodium)	Topical gel	Analgesic, anti-inflammatory	Zyduscadila (Approved)	Topical
BF-200 ALA-gel (5 Amino levulinic acid)	Topical gel	Actinic keratosis for photodynamic therapy	Biofrontera (Phase III trial)	Topical

ROA: Route of administration.

2.1.2 *Nanoemulsions*

NEs are thermodynamically/thermokinetically stable transparent nanodispersions of oil and water stabilized by an interfacial film of surfactant usually in combination with cosurfactant molecules with droplet size of less than 100 nm [19].

It has been reported that NEs prepared using LEE techniques are thermodynamically stable whereas those prepared using high-energy emulsification (HEE) techniques are thermokinetically stable [18-26].

2.1.3 *Solid lipid nanoparticles*

SLNs are lipid-based colloidal nanovehicles made up of biodegradable fats with a particle size of less than 1000 nm (typically 5 – 500 nm). These nanovehicles have been extensively used in drug delivery of both hydrophilic as well as hydrophobic drugs [27,28].

2.1.4 *Nanostructured lipid carriers*

NLC are colloidal systems made up of solid lipids and liquid lipids (oils) with particle size of 40 – 1000 nm [14].

2.1.5 *Nanocapsules*

Nanocapsules are nanosized, hollow, spherical-shaped colloidal systems with a core shell structure, where the core acts as a liquid reservoir for drugs or molecules and shell acts as a protective membrane. The particle size of nanocapsules is usually less than 1000 nm. They can encapsulate small amount of pharmaceuticals, enzymes, catalyst, and other materials [29].

2.1.6 *Liposomes*

Liposomes are spherical vesicles made up of amphiphilic phospholipids and cholesterol, which self associate into bilayers to encapsulate an aqueous inner part with vesicle size of 50 – 800 nm. Liposomes are of three types viz. multilamellar vesicles, large unilamellar vesicles, and small unilamellar vesicles [17,27].

2.1.7 *Transferosomes*

Transferosomes are the modified form of liposomes. These are complex vesicles optimized to attain extremely flexible and self-regulating membranes, which makes the vesicles very deformable. These are more elastic than ordinary liposomes and niosomes [30].

2.1.8 *Niosomes/proniosomes*

Niosomes are nonionic surfactant vesicles which are obtained by hydration of synthetic nonionic surfactant with or without the incorporation of cholesterol or other lipids with vesicle size ranging from micrometers to nanometers. Niosomes are physically more stable than liposomes. Proniosomes are dry formulation of water soluble vesicles that are coated with nonionic surfactant and can be rehydrated to form niosomal dispersion immediately before use on brief agitation in hot aqueous media [16,31].

2.2 Polymer-based nanovehicles

2.2.1 *Polymeric nanoparticles*

Polymeric nanoparticles are solid colloidal transporters made up of biodegradable materials such as proteins or polymers with particle size of 10 – 1000 nm. Polymeric nanoparticles have been extensively used in drug delivery [27].

2.2.2 *Nanocrystals*

Nanocrystals are colloidal dispersion of drug crystals stabilized by surfactants or polymers with physical dimensions of 2 – 100 nm. Nanocrystals have also been reported to have great potential for drug delivery [15].

2.2.3 *Polymeric micelles*

Polymeric micelles are nanosized, spherical colloidal drug carriers in which particles are structured by one hydrophilic exterior (shell) and one hydrophobic interior (core) with particle size of less than 50 nm. Polymeric micelles are divided into two main categories: hydrophobically assembled micelles and polyion-complex micelles [27,32].

2.2.4 *Dendrimers*

Dendrimers are a unique class of polymeric macromolecules of nanometer dimensions (usually 10 – 100 nm) with a highly branched structure and globular shape. They are synthesized via divergent or convergent synthesis by a series of controlled polymerization reactions [27].

3. Preparation and characterization of NEs

The basic objectives of the NE preparation are to achieve the droplet size in nanometer range and another is to provide stabilizing composition conditions. Generally two methods, utilizing HEE or LEE techniques, are used for the preparation of NEs [24,33-36]. HEE methods include high-pressure homogenization (HPH), microfluidization, and ultrasonication. HPH and microfluidization can be conducted at the industrial scale whereas ultrasonication is primarily used at bench level. Although HEE methods are very effective in the reduction of droplet size, they are unsuitable for labile compounds and macromolecules including proteins and peptides. For these reasons, LEE methods are generally preferred for preparation of NEs. LEE methods include spontaneous emulsification (aqueous phase/oil phase titration method), the solvent diffusion method, and the phase-inversion temperature (PIT) method [18-26]. **Table 2** lists the various techniques and their descriptions for preparation of NEs.

It is of utmost importance to have an in-depth knowledge about various characteristic properties that nanoemulsion must possess for their successful commercial exploitation. But complete characterization of nanoemulsion is quite difficult due to their complexity, different structures and components involved in these systems as well as the limitations associated with these techniques. Over the years, nanoemulsions have been characterized by different techniques but a complementary method is generally required to substantiate the results and overcome the limitations of the method used. At the macroscopic level viscosity, conductivity, and dielectric methods provide useful information. **Table 2** lists the various techniques and their applications for characterization of nanoemulsions for TDDS [11,18-24].

4. Technical requirements for a NE to achieve dermal delivery of drugs

Technical requirements that need to be achieved for dermal delivery but not transdermal delivery of drugs from NEs can be summarized as follows [37-39]:

1) The oil/water partition coefficient of drug should be very low, i.e., its lipophilicity should be low.
2) The molecular size of drugs should be greater than 500 daltons, because if the molecular size of the drug is low, it can easily penetrate the stratum corneum and the drug will enter into the systemic circulation.
3) Molecular weight of drug should be larger.
4) The percutaneous absorption (absorption of drug through the skin) of drugs should be very low.
5) The permeation rate and permeability coefficient of drug through the skin should be very low.
6) The systemic bioavailability of drugs should also be low.

5. Nanoemulsions as vehicles for *in vitro*/*in vivo* dermal delivery of hydrophobic compounds

An overview of *in vitro/in vivo* studies using NEs as vehicles for dermal delivery of hydrophobic compounds is given in **Table 3**.

Mitri *et al.* incorporated natural carotenoid lutein in different nanovehicles such as SLN, NLC and a NE for its dermal delivery. These nanovehicles were prepared by the HPH method. The mean particle size of these nanovehicles ranged between 150 and 350 nm. *In vitro* release studies were performed using a membrane-free model. The highest release in 24 h was observed for the NE formulation (19.5%). *In vitro* penetration studies were also performed using a cellulose acetate membrane which also showed highest values for the NE (60% after 24 h). Permeation studies with fresh pig ear skin showed that no or very little lutein (0.4% after 24 h) permeated from SLN and NLC that indicated that lutein is not systemically absorbed. These studies showed that the lipid nanovehicles are potential dermal vehicle for lutein [39].

Mahdi *et al.* developed a NE cream for dermal/topical delivery of 30% ethanolic extract derived from local *Phyllanthus urinaria* for skin antiaging. Palm kernel oil esters (POEs)-based NE of *P. urinaria* extract were prepared using a spontaneous emulsification method and characterized in terms of particle size, zeta potential, and rheological properties. The *in vitro* release profile of the extract was

Table 2. Preparation and characterization of nanoemulsions.

Preparation techniques	Description
High pressure homogenization (HPH)	HPH is performed by applying a high pressure over the system of oil phase, aqueous phase, and surfactants, making use of a high-pressure homogenizer or a piston homogenizer to produce nanoemulsions of extremely low droplet size. Drawbacks associated with these homogenizers are poor productivity, component deterioration due to difficult mass production, and generation of much heat (**Figure 1**). Using this technique, only o/w liquid nanoemulsions containing less than 20% oil phase can be prepared (**Figure 2**)
Microfluidization	Microfluidization is a patented mixing technology, which makes use of a device called microfluidizer for the production of nanoemulsions. This device uses a high-pressure positive displacement pump (500 – 20000 psig), which forces the product through the interaction chamber. From the interaction chamber the product flows through the small channels called 'microchannels' onto an impingement area resulting in very fine particles of sub-micron range
Ultrasonication	Ultrasonication provides another route for the preparation of nanoemulsions. In this method, the droplet size of conventional emulsions or even nanoemulsions gets reduced with the help of sonication mechanism
Spontaneous emulsification	This method is the simplest method for the preparation of nanoemulsions. They can be prepared simply by blending in right proportions oil, water, surfactant and cosurfactant with mild agitation. The order of mixing the components is generally considered not to be critical since they are formed spontaneously. Large transitory fluctuations in interfacial tension can occur during the nanoemulsification process as the components arrange themselves in such a way that the resulting interfacial and bulk microstructures lead to an overall decrease in the free energy
Phase inversion temperature (PIT)	In this method, a fine dispersion is obtained by chemical energy as a result of phase transitions taking place. The phase transitions are produced by varying the composition at constant temperature or by varying the temperature at constant composition
Characterization techniques	**Description**
Viscosity measurements	These measurements are suitable to evaluate flow behavior of nanoemulsion
Conductivity measurements	It determines the type of nanoemulsion and detects the phase inversion phenomenon
Dielectric measurements	These are powerful means of probing both structural and dynamic features of nanoemulsions.
Phase behavior studies	Phase diagram are used to evaluate these behavior
Nuclear magnetic resonance (NMR) studies	The structure and dynamics of nanoemulsions can be studied by NMR techniques, self-diffusion measurements using different tracer techniques, generally radio labeling, supply information on the mobility and nanoenvironment of the components. The Fourier transform pulsed-gradient spin-echo (FT-PGSE) techniques use the magnetic gradient on the samples and it allows simultaneous and rapid determination of the self diffusion coefficients of many components
Electron microscopic studies: FFTEM & TEM	Freeze fracture transmission electron microscopy (FFTEM) and transmission electron microscopy (TEM) are the most important techniques for the study of nanostructures because it directly produces images at high resolution and it can capture any coexistent structure and nanostructure transitions
Scattering techniques: SAXS, SANS & PCS	Scattering methods that have been employed in the study of nanoemulsions include small angle X-ray scattering (SAXS), small angle neutron scattering (SANS) and static light scattering & dynamic light scattering or photon correlation spectroscopy (PCS). All these techniques are used to determine droplet size distribution
Interfacial tension measurement	The formation and properties of nanoemulsions can be studied by measuring the interfacial tension. Spinning-drop apparatus can be used to measure the ultra low interfacial tension
Thermal characterization	Differential scanning calorimetry (DSC) is used for thermal characterization
Other methods	Dye solubility and dilution test, plane polarized light microscopy, refractive index, and zeta potential measurements

Table 3. Overview of nanoemulsions as vehicles for *in vitro/in vivo* dermal delivery of hydrophobic compounds.

Compounds	Nanoemulsion components				Membrane/Skin species	Purpose	Ref
	Oil phase	Surfactant	Cosurfactant	Aqueous phase			
Lutein	Miglyol-812	Plantacare-810	-	Water	Cellulose acetate, Pig	Enhance dermal delivery	[39]
Phyllanthus urinaria	POEs	Tween-80	Span-80	PBS	Cellulose acetate	Enhance dermal delivery	[40]
RBO	RBO	PEG-30 castor oil	Sorbitanoleate	Water	Chorioallantoic, human	Evaluate irritation potential and moisturizing activity	[41]
Clobetasol propionate	MCT	Polysorbate-80	Span-80	Water	Cellulose acetate, rat	Enhance dermal delivery and dermatological efficacy	[42]
Estradiol, progesterone, cyproterone acetate, finasteride	Eucalyptus oil	Brij-30	Ethanol	Water	Porcine	Enhance permeation and stability	[43]
Fludrocortisone acetate	PCL liquid	Lecithin E-80	Potassium sorbate	Water	Porcine	Enhance permeability	[44]
Ketoprofen	POEs	Tween-80	-	Water	Methyl cellulose acetate	Enhance dermal delivery	[45]
	POEs	Tween-80	-	Water	Rat	Evaluate anti-inflammatory and analgesic effects	[46]
MS	Soybean oil	Soybean lecithin, Tween-80, polloxamer-407	PG	Water	Rat	Enhance dermal delivery	[47]
Prednicarbate	Lipoid-E80, α-tocopherol, eutanol	Tween-80	-	Water	-	Enhance stability	[48]
Foscan®	Miglyol-812	Epikuron-170	Poloxamer-180	Water	Rat	Evaluate diffusional flux and anticancer potential	[49]
	Miglyol-812	Epikuron-170	Span-80	Water	Pig	Enhance permeability and anticancer potential	[50]
Ceramides	Triglycerides	Phosphatidycholine	-	Water	-	Characterization and stability evaluation	[53]
Genistein	MCT, ODD	Lecithin	-	Water	Pig	Assess topical delivery	[54]
LA	Miglyol-812	Pluronic-F68	-	Water	Synthetic membrane	Enhance topical delivery and antioxidant activity	[55]
Quercetin, MQ	ODD	Lecithin	Cetrimide	Water	Pig	Assess topical delivery	[56]
Propolis-lycopene	-	-	-	Water	Guinea pig	Evaluate skin irritation and anti-inflammatory effects	[57]

8N8: Nonionic surfactant; LA: α lipoic acid; MCT: Medium chain triglyceride; MQ: 3-O-Methylquercetin; MS: Methyl cellulose; ODD: Octyldodecanol; PBS: Phosphate buffer saline; PG: Propylene glycol; POEs: Palm oil esters; RBO: Rice bran oil.

Table 3. Overview of nanoemulsions as vehicles for *in vitro/in vivo* dermal delivery of hydrophobic compounds (continued).

Compounds	Nanoemulsion components				Membrane/Skin species	Purpose	Ref
	Oil phase	Surfactant	Cosurfactant	Aqueous phase			
Curcumin	MCT	Tween-20	-	Water	Mouse	Enhance anti-inflammatory effects	[59]
Paclitaxel	Labrafil	Labrasol	Ethanol	Water	Rat	Enhance oral and dermal bioavailability	[60]
8N8	Soybean oil	Triton-X100	-	Water	Bacteria, virus, fungi	Enhance topical biocidal activity	[61]
Tocopherol	Soybean oil	Polysorbate-80	Phosphatydicholine	Water	Mouse	Enhance anti-inflammatory activity and bioavailability	[62]

8N8: Nonionic surfactant; LA: α lipoic acid; MCT: Medium chain triglyceride; MQ: 3-O-Methylquercetin; MS: Methyl cellulose; ODD: Octyldodecanol; PBS: Phosphate buffer saline; PG: Propylene glycol; POEs: Palm oil esters; RBO: Rice bran oil.

evaluated using Franz diffusion cells from cellulose acetate membrane and the antioxidant activity of the extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. *In vitro* release of the extract from NE formulations showed DPPH radical scavenging activity. Therefore, these formulations could neutralize reactive oxygen species (ROS) and counteract oxidative injury induced by ultraviolet radiation and thereby ameliorate skin aging [40].

Bernardi *et al.* developed a rice bran oil (RBO) NE using the LEE method and evaluated their physical stability, *in vitro* irritation potential and *in vivo* moisturizing activity. The developed NE was found to be stable over the time course of this study. *In vitro* assay indicated that this formulation has a low irritation potential. *In vivo* studies on human skin showed that the NE improved the skin's moisture and maintained normal skin pH values. Overall these results indicated that developed NE has potential to be a useful tool to treat skin diseases, such as atopic dermatitis and psoriasis [41].

Fontana *et al.* developed dermal hydrogels containing clobetasol propionate-loaded lipid-core nanocapsule (HG-CP-NC) or NE (HG-CP-NE) to evaluate the influence of the polymeric wall on *in vitro* drug release and *in vivo* therapeutic efficacy in a model of contact dermatitis after topical application in rats. The best *in vitro* drug release control was obtained for HG-CP-NC as compared to the HG-CP-NE and the hydrogels containing nonencapsulated drug. The nanoencapsulation of clobetasol led to a better control of the drug release from the HG-CP-NC. A significant increase in nucleoside triphosphate diphosphohydrolase (NTPDase) activity was observed in lymphocytes for the group treated with HG-CP-NC every other day as compared to the group treated with HG-CP every day in the *in vivo* model of contact dermatitis. Enhanced *in vitro* drug release and significant increase in NTPDase activity indicated that nanomedicine provided better *in vivo* dermatological efficacy [42].

Birus *et al.* developed and evaluated the skin permeation and the chemical stability of 17-β-estradiol, progesterone, cyproterone acetate, and finasteride incorporated into a eucalyptus oil NE system. Drug stability in each NE system was monitored by analyzing the steroid hormones content in the different formulations over a period of 6 weeks. Results of these studies showed enhanced skin permeation and stability of these steroidal drugs through porcine skin [43].

Klang *et al.* investigated the effects of cyclodextrins (CDs) on the skin permeation of dermally applied fludrocortisone acetate from a NE. The role of critical diffusion cell parameters such as the dose of application, occlusive conditions, the nature of the receptor medium, and the skin thickness were investigated in the present study. The results of this study showed that significant enhancement in skin permeation rates of fludrocortisone acetate were indeed caused by 1% w/w of γ-CD at both finite and infinite dose conditions. It was concluded that the full permeation enhancement potential of the CD be realized at infinite dose conditions while preserving the formulation structure [44].

Sakeena *et al.* investigated the potential of NEs for dermal/topical delivery of ketoprofen. POEs, a newly-introduced oil, was used as the oil phase for the preparation of NE. NE were prepared by spontaneous emulsification method and characterized by laser scattering spectroscopy (LSS) and transmission electron microscopy (TEM). *In vitro* drug release profile of ketoprofen NE was determined through methyl cellulose acetate membrane using Franz diffusion cell. *In vitro* drug release profile showed significant release of ketoprofen through methyl cellulose acetate membrane. These preliminary studies showed that NEs formulated using POEs have great potential for topical delivery of ketoprofen [45]. In other significant research, Sakeena *et al.* evaluated anti-inflammatory and analgesic effects of a topically applied NE system of ketoprofen on rats. These *in vivo* effects of ketoprofen NE were also compared with marketed Fastum® gel. Results of these studies showed no significant difference in anti-inflammatory and analgesic effects of ketoprofen from NE and marketed Fastum® gel. From these studies, it was concluded that the developed NE has good potential for topical delivery of ketoprofen [46].

Mou *et al.* investigated a hydrogel-thickened nanoemulsion (HTN) system for dermal/topical delivery of active hydrophobic molecules. HTN was prepared to deliver the lipophilic mixture of 5% camphor, 5% menthol, and 5% methyl salicylate for topical therapy of arthritis, minor joint, and muscle pain using soybean oil as the oil phase, soybean lecithin, Tween 80, and poloxamer 407 as the surfactants, propylene glycol as the cosurfactant and carbomer 940 as a thickening agent. The HTN system was found to combine the o/w microstructure of NE with the gel network of the hydrogel. This system was found to have small average diameters and good long-term stability. The *in vitro* permeation profile of HTN was evaluated by diffusional apparatus using rat skin. The permeation rates of camphor, menthol and methyl salicylate from the optimal HTN formulation were found to be 138.0 ± 6.5, 63.6 ± 3.3, and 53.8 ± 3.2 μg/cm^2/h, respectively, and showed significant advantages over the control gel. These studies showed that the HTN with good stability and powerful permeation enhancing ability, and suitable viscosity might be a promising prospective vehicle for topical delivery of lipophilic drugs [47].

Baspinar *et al.* developed and investigated a physically and chemically stable positively charged NE of prednicarbate as a vehicle for the treatment of atopic dermatitis. Phytosphingosine (PS) was used to obtain the positive charge and to provide supportive properties for the restoration of damaged skin. A positively-charged NE was produced using the HPH method. The concentrations of PS, oil phase, and the emulsifiers were also optimized in the study. The production of the NE was optimized by evaluating the influence of homogenization cycles, homogenization pressure, production temperature, and type of homogenizer with respect to particle size, physical stability, and chemical stability of prednicarbate. It was optimized so that the NE could be produced at elevated temperatures with low homogenization pressures but higher numbers of homogenization cycles. These findings showed that the optimal formulation was not only characterized by the smallest particle size and the best stability, but also due to the fact that the smallest particles do not have the highest chemical stability. Further studies would investigate the release and the skin permeation pattern of the optimized formulation [48].

Primo *et al.* evaluated a magnetic nanoemulsion (MNE) of Foscan® (temoporfin) in terms of skin permeation and retention *in vitro* assay for dermal/topical application in photodynamic therapy (PDT) of skin cancer. The association of colloidal nanovehicles with biocompatible magnetic fluids resulted in a new drug delivery system (DDS) for the application in PDT and magnetic hyperthermia treatment. They investigated the skin permeation using Foscans® as a photosensitizer loaded in MNE using a Franz diffusion cell. The diffusional flux of Foscans® was found to be enhanced by MNE. The developed MNE was found to work in a synergistic manner with an expected enhancement in tumor damage with minimum drug doses, based on heat dissipation and/or light photosensitization. They investigated this system in a biological skin model in biomimetic conditions. The retention studies showed that the concentration of the Foscans® in deep tissue layers was significantly higher in the presence of magnetic nanovehicles, making possible its topical application in future skin cancer PDT protocols and/or hyperthermia activation in synergic procedures [49]. Primo *et al.* in another significant research work evaluated the *in vitro* and photophysical properties of Foscan using a biodegradable o/w NE. *In vitro* biological behavior studies were carried out in a mimetic biological environment protocol based on an animal model. After topical application of NE in a skin animal model, the flux of Foscan/NE into the skin layers (stratum corneum and epidermis + dermis) was evaluated using Franz diffusion cells. The photodynamic potential of the drug loaded NE was studied by steady-state and time-resolved spectroscopic techniques. Results showed that the photophysical properties of PS were maintained after its loading into the NE as compared to the homogeneous organic medium. Finally these studies indicated that the NE can be potentially applied as a DDS for Foscan *in vivo*, as well as in future clinical applications involving topical skin cancer PDT [50]. In another significant study, Primo *et al.* described the preparation of a nanovehicle for the controlled release of a photosensitizer compound for skin wound healing treatment and applicable to other skin diseases. A biological model was used as an *in vitro* skin equivalent based on a three-dimensional culture of fibroblasts and mesenchymal stem cells. Results of this study showed that it is possible to use the photomodulation process to control the wound healing in a scratching process and to induce the biomolecule releases. Overall results of this study showed a potential application in wound healing processes based on phototherapy and nanotechnology [51].

Aubrun *et al.* prepared NE with a high shear device, which was less constraining than spontaneous emulsification procedures. They found that NE were easily acceptable in skin care due

to their good sensorial properties (rapid penetration and merging textures) and their biophysical properties (especially their hydrating power) [52].

Deli *et al.* investigated the possibility of using the benefits of nanotechnology in the efficient topical delivery of ceramides formulated as NE or SLN. The physicochemical characteristics and stability of these nanovehicles incorporating ceramides were investigated. Their morphology was examined under a scanning electron microscopy (SEM) and the interactions of the components of nanovehicles were studied by differential scanning calorimetry (DSC) analysis. The results indicated that the NE can incorporate a high percentage of ceramides giving more homogeneous particle distributions of spherical-shaped nanoparticles and they maintained their characteristics over the time. On the contrary, SLN incorporation of ceramide led to the formation of rod-like nanoparticles deteriorating the homogeneity of the particle distribution. The results demonstrated that NE may be the more suitable vehicle as compared to SLN [53].

Silva *et al.* described the physicochemical properties and *in vitro* skin permeation profile of genistein from NE. NE formulations were prepared by spontaneous emulsification method using egg lecithin, medium chain triglycerides (MCT) or octyldodecanol (ODD) and water. This method was found to be yielded monodisperse emulsions with mean droplet sizes in the range of 230 – 280 nm. The addition of genistein in the oil phase did not change the physicochemical properties of NE. The amount of genistein incorporated in NE formulations was close to 100%. In order to investigate the location of genistein in the NE, its solubility in both MCT and ODD oils was determined. The low solubility (235.3 μg/ml and 137.9 μg/ml for MCT and ODD, respectively) of genistein in both oils clearly suggested a possible role of egg lecithin on genistein encapsulation in NE. Therefore, DSC experiments were performed to investigate the effects of egg lecithin on thermal properties of genistein. DSC thermograms of genistein/egg lecithin mixture showed different peak as compared to pure genistein and egg lecithin suggested some interaction between phospholipids of egg lecithin and genistein. These interactions could be related to the high incorporation efficiency of genistein into NE. These results indicated that egg-lecithin could play an important role in the encapsulation of genistein into NE. *In vitro* skin permeation of genistein from NE was evaluated using pig ear skin in Franz diffusion cells. These results showed a slow skin permeation profile for genistein from NE which could be open interesting perspectives for the topical administration of genistein [54].

Ruktanonchai *et al.* formulated and evaluated α-lipoic acid (LA) in the form of SLN, NLC, and NE. These nanovehicles were characterized in terms of physical and biological properties. Mean particle size of NE, NLC, and SLN were found to be less than 150 nm with narrow size distribution. Zeta potential was found to be in the range of -25 to -40 mV for all nanovehicles. Disc and spherical-shaped structures of nanoparticles were observed using cryo-SEM. All the formulations were found to be physically stable at room temperature (25°C). Faster drug release was observed from NE compared with that of SLN and NLC. Antioxidant activity studies demonstrated that all LA-loaded nanovehicles expressed antioxidant activity at a similar magnitude as pure LA. These results suggested that chosen compositions of lipid nanovehicles play an important role on biological activity of LA. Both SLN and NLC indicated their potential as alternative vehicles for topical administration of LA [55].

Fasolo *et al.* described the physicochemical properties and the *in vitro* skin permeation profile of quercetin (Q) and 3-O-methylquercetin (MQ) from lipid NEs. NE formulations were prepared by spontaneous emulsification method using ODD, egg lecithin, water, and cetyl trimethyl ammonium bromide (CNE). This procedure was found to yield monodisperse NE with a mean droplet size of approximately 200 – 300 nm. These NEs were further characterized in terms of zeta potential, surface tension, and morphology using TEM. The *in vitro* skin permeation studies were carried out using ear pig skin mounted in Franz diffusion cells. Results of these studies showed a slow permeation profile of both Q and MQ from prepared NE. These results indicated that NE could be the open interesting perspectives for the topical administration of the flavonoids Q and MQ [56].

Butnariu *et al.* evaluated the harmlessness of propolis–lycopene extract NE through evaluation of skin level changes and anti-inflammatory action. The developed NE formulations did not reveal a deteriorating effect on tissues. They showed a better therapeutic efficacy as compared to standard suspension. Developed formulations were found to be nonirritant to the skin. This study demonstrated that a propolis and lycopene extract NE could confer better therapeutic effects than those of the conventional formulations. The data of the present study suggested that the administration of propolis and lycopene aqueous extract NE is safe. The preparation could be useful for further preclinical studies for targeted medical therapy [57].

Gholam *et al.* compared the pain intensity using 5-aminolaevulinic acid methylester (MAL) or 5-aminolaevulinic acid NE (BF-200-ALA). A total of 173 patients with 965 treated areas were enrolled in this study. All patients had multiple actinic keratosis (AKs) and received an extensive treatment of the photodamaged area. The number of PDT treatment interruptions was recorded. PDT with MAL led to a lower mean score of pain, a lower number of treatment interruptions, and a lower amount of patients experiencing severe pain compared to PDT with BF-200-ALA. The data of this study showed that PDT using MAL is less painful than PDT using BF-200-ALA [58].

Wang *et al.* evaluated enhanced anti-inflammatory effects of natural anti-inflammatory drug curcumin from o/w NE. Curcumin-loaded o/w NE were prepared by high-speed and HPH method using MCT as oil and Tween 20 as emulsifier with mean droplet sizes ranging from 79.5 to 618.6 nm. The enhanced anti-inflammatory effects of curcumin encapsulated o/w NE were evidenced by the mouse ear inflammation model. There was a 43% or 85% inhibition in inflammation of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced edema

of mouse ear for 618.6 nm and 79.5 nm NE, respectively, but a negligible effect was found for 1% curcumin in 10% Tween-20 water solution [59].

Khandavilli and Panchagnula investigated *in vivo* pharmacokinetic performance of paclitaxel (PCL)-NE in order to achieve penetration of PCL into deeper skin layers. Further, the same formulation was explored for peroral bioavailability enhancement of PCL. They concluded that developed NE formulation was safe and effective for both peroral and dermal delivery of PCL [60].

Hamouda1 *et al.* tested a novel nonionic surfactant NE designated 8N8 for its biocidal activity. One percent 8N8 produced effective bactericidal activity, virucidal activity, and fungistatic activity against all tested strains of bacteria, virus, and fungi, respectively, in 15 min. The rapid and nonspecific inactivation of vegetative bacteria and enveloped viruses made 8N8 a potential candidate for use as a topical biocidal agent [61].

Kuo *et al.* investigated topical anti-inflammatory effects and bioavailability of MicroFluidizer Processor®-based NE of an antioxidant synergy formulation (ASF), containing delta, alpha and gamma-tocopherol in mice. Croton oil was applied to induce inflammation in all animals. The mice plasma and ear lobes were collected and frozen for bioavailability and cytokine analysis, respectively. The ASF NE of alpha, delta or gamma tocopherol were found to reduce auricular thickness significantly as compared to control (57, -57, and -71%, respectively) and blank NE (-50, -50, and -67%, respectively). Bioavailability of gamma and delta tocopherol was dramatically enhanced (2.2 and 2.4-folds) with the NE as compared to the suspensions. They finally concluded that NE of ASF containing gamma, alpha and delta tocopherol have enhanced anti-inflammatory effects and increased bioavailability with gamma tocopherol, in particular compared to their suspensions [62].

6. Nanoemulsions as vehicles for *in vitro*/*in vivo* transdermal delivery

An overview of *in vitro/in vivo* studies using NE as vehicles for transdermal delivery of hydrophobic compounds is given in **Table 4**. Most of these *in vitro/ex vivo* or *in vivo* investigations were performed using Franz diffusion cells. Various animal models were used to study transdermal delivery.

Zhang *et al.* prepared and evaluated 3,5-dihydroxy-4-isopropylstilbene (DHPS) NE by LEE method using polyoxyethylenated castor oil (EL-40) as the surfactant, ethanol as the cosurfactant, and isopropyl myristate (IPM) as the oil phase. Developed DHPS NE was found to have low viscosity and spherical droplets with uniform distribution. The NE showed significant improvement in the transdermal permeation of DHPS and could become a favorable new dosage form for DHPS [63].

Sapgnul *et al.* developed and evaluated an innovative NE formulation of tricarboxylic calixarene dedicated to uranium skin decontamination. *ex vivo* experiments carried out in Franz diffusion cells using pig ear skin showed that the immediate application of the calixarene NE on a skin contaminated by a uranyl nitrate solution allowed uranium transcutaneous diffusion decrease of about 98% through intact and excoriated skins. Developed calixarene NE thus seems to be an efficient emergency system for uranium skin decontamination [64]. In another significant research, Spagnul *et al.* described the ability of calixarene NE to trap uranium and limit its transfer from the cutaneous contaminated site into the blood. Uranium percutaneous diffusion kinetics was performed using Franz diffusion cells through intact and excoriated pig ear skin biopsies, after or without application of the NE. The results of this study showed that prompt application of the calixarene NE allows a 94% and 98% reduction of the amount of uranium diffused, respectively, through intact and excoriated skin. Moreover, no accumulation of uranium or uranium-calixarene chelate was found in the different layers of skin. This study demonstrated the efficiency of the calixarene NE as a promising treatment for uranium cutaneous contamination [65].

Shakeel *et al.* investigated an o/w NE to increase skin permeation and anti-inflammatory effects of aceclofenac using a combination of Labrafil and Triacetin (2:1) as the oil phase, Tween-80 and Transcutol-P as surfactant and cosurfactant, respectively. Aceclofenac-loaded NE were prepared by the spontaneous emulsification method and characterized in terms of viscosity, droplet size, surface morphology, and refractive index. Transdermal flux of aceclofenac through rat abdominal skin was studied using Franz diffusion cells. The transdermal skin permeation profile of optimized NE was compared with that of aceclofenac conventional gel and NE gel. A significant increase in permeability parameters was observed with optimized NE formulation as compared to conventional aceclofenac gel and NE gel. The anti-inflammatory effects of optimized NE formulation F1 (containing 1 % aceclofenac, 10 % Labrafil, 5 % Triacetin, 35.33 % Tween-80, 17.66 % Transcutol-P, and 32 % distilled water) showed a significant increase in % inhibition value after 24 h application compared to aceclofenac conventional gel and NE gel in rats as shown in **Figure 3**. These results indicated that NE are potential vehicles for improved transdermal delivery of aceclofenac [66]. The skin permeation mechanism(s) of aceclofenac was also investigated using the same NE formulation. Fourier transform infrared (FTIR) spectral analysis, DSC thermograms, activation energy measurement, and histopathological examination were used to investigate the mechanism(s). FTIR spectra of skin treated with the nanoemulsion formulation showed breaking of the hydrogen bond network at the head of ceramides. The DSC thermogram of untreated rat skin (control) showed four endotherms at 34°C (T1), 82°C (T2), 105°C (T3), and 114°C (T4), respectively. However, the thermogram of the skin specimen treated with aceclofenac NE was found to be completely different from untreated skin. It was observed that both T2 and T3 endotherms completely disappeared or shifted to lower melting points in the thermogram of skin treated with NE. This clearly indicated that the components of the NE enhanced skin permeation of drugs through the extraction of

Table 4. Overview of nanoemulsions as vehicles for *in vitro/in vivo* transdermal delivery of hydrophobic compounds.

Compounds	Nanoemulsion components				Membrane/Skin species	Purpose	Ref
	Oil phase	Surfactant	Cosurfactant	Aqueous phase			
DHPS	IPM	EL-40	Ethanol	Water	Mouse	Enhance transdermal release	[63]
Calixarene	Paraffin oil	Tween-80	Span-80	Water	Pig	Decrease transcutaneous diffusion	[64]
	Paraffin oil	Tween-80	Span-80	Water	Pig	Decrease *ex vivo* uranium diffusion	[65]
Aceclofenac	Labrafil, Triacetin	Tween-80	Transcutol-P	Water	Rat	Enhance permeability and anti-inflammatory effects	[66]
	Labrafil, Triacetin	Tween-80	Transcutol-P	Water	Rat	Assess skin permeation mechanism	[67]
	Labrafil, Triacetin	Tween-80	Transcutol-P	Water	Rat	Enhance bioavailability	[68]
Celecoxib	Sefsol-218, Triacetin	Tween-80	Transcutol-P	Water	Rat	Assess skin permeation mechanism and bioavailability	[11]
	Sefsol-218, Triacetin	Cremophor-EL	Transcutol-P	Water	Rat	Assess skin permeation mechanism and bioavailability	[69]
	Sefsol-218, Triacetin	Cremophor-EL	Transcutol-P	Water	Rat	Enhance permeability	[70]
	Sefsol-218, Triacetin	Tween-80	Transcutol-P	Water	Rat	Enhance permeability and anti-inflammatory effects	[74]
	Sefsol-218, Triacetin	Cremophor-EL	Transcutol-P	Water	Rat	Enhance anti-inflammatory effects	[71]
Indomethacin	Labrafil	Tween-80	Transcutol-HP	Water	Rat	Enhance permeability	[72]
	Labrafil	Tween-80	Transcutol-HP	Water	Rat	Enhance anti-inflammatory effects	[73]
Capsaicin	BA	Ethanol, PG, n-butanol	-	Water	Rat	Enhance permeability	[75]
Fludrocortisone, flumethasone	Lipoid-S75, α-tocopherol	Sucrose laurate, polysorbate-80, PS	-	Water	Porcine	Enhance permeability	[76]
Progesterone	Lipoid-E80, α-tocopherol, PG	Sucrose esters	-	Water	Porcine	Enhance permeability	[77]
Ketoprofen	BA	Ethanol	Solutol	Water	Mouse	Enhance permeability	[78]
	POEs	Tween-80	-	Water	Rat	Evaluate permeability and skin irritation	[79]
Nabumetone	Soybean oil	Lecithin	1-*O*-alkylglycerol	Water	Rat	Enhance permeability	[80]
Nimesulide	MCT	Polysorbate-80	-	Water	-	Characterization	[81]
	MCT	Polysorbate-80	-	Water	Human	Assess permeability	[82]

BA: Benzyl alcohol; CA: Cetearyl alcohol; CP: Cetylpalmitate; DC: Dicaprylic carbonate; DHPS: 3,5 dihydroxy-4-isopropylstilbene; EL-40:polyoxyethylenated castor oil; GA: Glycyrrhetic acid; GS: Glyceryl stearate; IPA: Isopropyl alcohol; IPM: Isopropyl myristate; MCT: Medium chain triglyceride; OA: Oleic acid; OMC: Octylmethylcinnamate; PG: Propylene glycol; POEs: Palm oil esters; PS: Phytosphingosine; PVA: Polyvinyl alcohol.

Table 4. Overview of nanoemulsions as vehicles for *in vitro/in vivo* transdermal delivery of hydrophobic compounds (continued).

Compounds	Nanoemulsion components				Membrane/Skin species	Purpose	Ref
	Oil phase	Surfactant	Cosurfactant	Aqueous phase			
Amlodipine	OA	Tween-20	Transcutol-P	Water	Rat	Enhance permeability	[83]
Carvedilol	OA, IPM	Tween-80	Transcutol-P	Water	Rat	Enhance permeability and solubility	[84]
Domperidone	OA	Polysorbate-20	Carbitol	Water	Rat	Enhance percutaneous absorption and bioavailability	[85]
	Caproyl-90	Tween-20	Carbitol	Water	Rat	Enhance permeability	[86]
Ropinirole	Caproyl-90	Tween-20, Cremophor-EL	Carbitol	Water	Rat	Assess pharmacokinetic, biochemical and mechanistic studies	[87]
Lidocaine	BA	Tween-80	Ethanol	Water	Rat	Enhance permeation rate	[88]
Tetracaine	Hexadecane	Synperonic-A7	-	Water	Human	Assess permeation	[89]
Bentex®	IPM	Labrasol	Plurol	Water	Rat	Enhance Cutaneous delivery	[90]
GA	Ceteareth-12, 20, GS, CA, CP	DC	-	Water	Human	Enhance transdermal delivery and anti-inflammatory effects	[91]
Granisetron hydrochloride	IPM	Tween-85	Ethanol	Water	Rat	Enhance permeability	[92]
Tocopherol	OA	Tween-80	Ethanol	Water	Mouse	Decrease tumor growth	[93]
	Canola oil	Polysorbate-80	-	Water	Hamsters	Enhance bioavailability	[94]
Ceremides	Cholesterols	Lecithin	-	Water	Human	Enhance skin hydration and elasticity	[95]
OMC	2-Butanone	Sucrose esters	PVA	Water	Human	Enhance percutaneous absorption	[96]

BA: Benzyl alcohol; CA: Cetearyl alcohol; CP: Cetylpalmitate; DC: Dicaprylic carbonate; DHPS: 3,5 dihydroxy-4-isopropylstilbene; EL-40:polyoxyethylenated castor oil; GA: Glycyrrhetic acid; GS: Glyceryl stearate; IPA: Isopropyl alcohol; IPM: Isopropyl myristate; MCT: Medium chain triglyceride; OA: Oleic acid; OMC: Octylmethylcinnamate; PG: Propylene glycol; POEs: Palm oil esters; PS: Phytosphingosine; PVA: Polyvinyl alcohol.

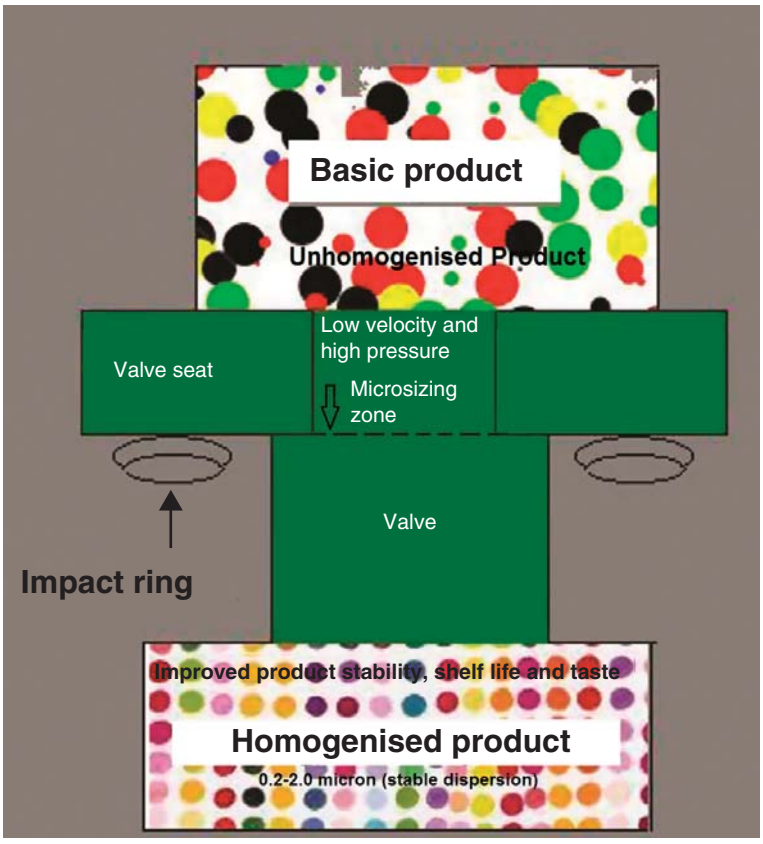

Figure 1. High pressure homogenization method for production of nanoemulsions.

subcutaneous lipids. The NE formulation also decreased the protein endotherm T4 to a lower melting point, suggesting keratin denaturation and a possible intracellular permeation mechanism. Therefore, it was concluded that intracellular transport could be a possible mechanism of permeation enhancement. The significant decrease in activation energy for aceclofenac permeation suggested that the stratum corneum lipid bilayers were significantly disrupted, since they are principal barriers to transport. Photomicrographs revealed the disruption and extraction of lipid bilayers [67]. In another important study, Shakeel *et al.* compared the pharmacokinetic profile (bioavailability) of aceclofenac by transdermal and oral application. NE, NE gel, and marketed tablet (Aceclofar®) of aceclofenac were subjected to pharmacokinetic studies on rats. The *in vivo* absorption of aceclofenac by transdermally applied NE and NE gel was found to be 2.95- and 2.60-fold increase in bioavailability as compared to marketed tablet formulation as indicated in **Figure 4**. These studies indicated that the NE can be successfully used as potential vehicle for enhancement of bioavailability of aceclofenac [68]. In another significant study, Shakeel *et al.* assessed skin permeation mechanism and bioavailability of two NE formulations of CXB using combination of Sefsol-218 and Triacetin (1:1) as oil phase, Tween-80 or Cremophor-EL as the surfactant, and Transcutol-P as cosurfactant. These mechanisms were evaluated in terms of FTIR spectral analysis, DSC thermograms, activation energy measurement and histopathological examinations. Results of these studies also indicated that NE can be successfully used for enhanced transdermal permeation of CXB both *in vitro* as well as *in vivo* [11,69]. The *ex vivo* skin permeation profile of CXB was also examined using a 1:1 combination of Sefsol-218 and Triacetin (1:1) as the oil phase with Cremophor-EL and Transcutol-P as surfactant and cosurfactant, respectively. An enhanced skin permeation profile for CXB was obtained by the optimized NE as compared to a conventional CXB gel or NE gel [70]. Shakeel *et al.* also investigated the enhanced anti-inflammatory effects of CXB from a transdermally applied NE. The anti-inflammatory effects of an optimized NE formulation were compared with those of conventional CXB gel and NE gel in rats. The % inhibition value after 24 h application was found to be significant for NE formulation C2 (containing 2 % CXB, 7.5 % Sefsol-218, 7.5 % Triacetin, 17.5 % Cremophor-EL, 17.5 % Transcutol-P, and 50 % distilled water) was 85.4% compared with CXB gel and NE gel. These results showed that NE could be successfully used to enhance the anti-inflammatory effects of CXB [71]. In another important study, Shakeel *et al.* evaluated the potential of true NE for

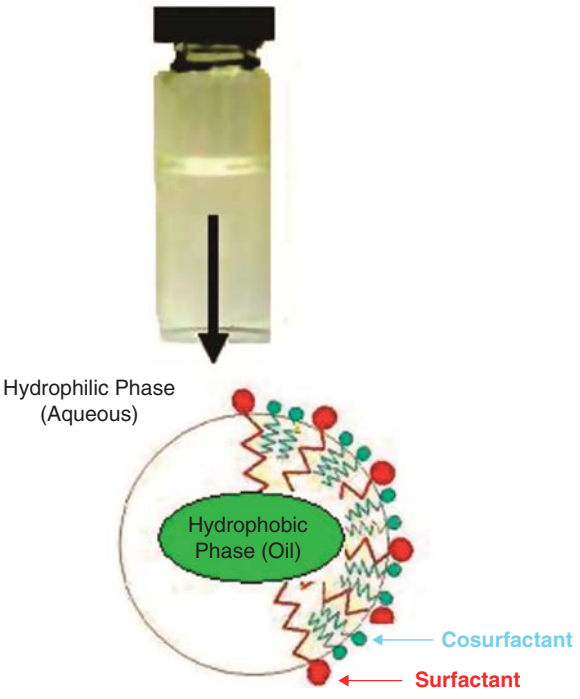

Figure 2. An oil-in-water (o/w) type nanoemulsion with structural droplets demonstration.

transdermal delivery of indomethacin. The *ex vivo* skin permeation studies were performed in Franz diffusion cell using rat skin. The *ex vivo* skin permeation profile of optimized formulation was compared with Indobene gel and NE gel. Significant increase in steady-state flux (J_{ss}), permeability coefficient (K_p) and enhancement ratio (E_r) was obtained in NE formulations. The J_{ss} and K_p for optimized NE formulation were found to be 73.96 ± 2.89 μg/cm^2/h and 1.479 × 10^{-2} ± 0.289 × 10^{-2} cm/h, respectively, which were significant compared with Indobene gel and NE gel. E_r was found to be 7.88 in optimized formulation F6 (containing 0.5 % indomethacin, 5 % Labrafil, 33.75 % Tween-80, 11.25 % Transcutol-HP, and 50 % distilled water). These results suggested that NE can be used as potential vehicles for improved transdermal delivery of indomethacin [72]. Shakeel *et al.* also enhanced anti-inflammatory effects of indomethacin from a transdermally applied NE. The anti-inflammatory effects of an optimized nanoemulsion formulation were compared with those of marketed Indobene gel. The % inhibition value after 12 h application was significant for optimized formulation F6 (i.e., 83% compared with marketed Indobene gel). These results suggested that NE can be successfully used to enhance the anti-inflammatory effects of indomethacin [73].

Baboota *et al.* also developed and investigated o/w NE to enhance the skin permeation and anti-inflammatory effects of CXB using combination of Sefsol-218 and Triacetin (1:1) as oil phase, Tween-80 as surfactant, and Transcutol-P as cosurfactant. *ex vivo* skin permeation of CXB was performed in modified Keshary–Chein diffusion cell using rat skin as permeation membrane. The skin permeation profile of optimized NE was also compared with conventional gel and NE gel, all having same amount of CXB. Significant increase in the permeability parameters such as J_{ss}, K_p, and E_r was observed in optimized NE formulations as compared to CXB conventional gel and NE gel. The anti-inflammatory effects of NE formulation T2 (containing 2 % CXB, 5 % Sefsol-218, 5 % Triacetin, 25 % Tween-80, 25 % Transcutol-P, and 40 % distilled water) showed a significant enhancement in % inhibition after 24 h application compared to CXB conventional gel and NE gel on carrageenean-induced paw edema in rats. These results showed that NE are potential vehicle for transdermal delivery of CXB [74].

Li *et al.* prepared and evaluated the transdermal permeation of capsaicin NE. The capsaicin NE were optimized by simplex method with steady-state transdermal permeation rate as the investigation index using amphiphilic benzyl alcohol as oil phase, short chain alcohols (ethanol, propylene glycol, and n-butanol) as cosolvents. The area of NE was determined by the construction of ternary phase diagrams. The optimized NE was compared with the capsaicin ointment and the capsaicin hydrogel in terms of the steady-state transdermal rate in rats. The NE prepared using propylene glycol and ethanol were found to be equivalent in NE area and both were larger than that with n-butanol. Transdermal flux of the optimized capsaicin NE, capsaicin ointment, and capsaicin hydrogel were found to be 17.54, 2.78, and 7.35 μg/cm^2/h, respectively. These results indicated that NE has satisfactory transdermal permeation property for capsaicin [75].

Hoeller *et al.* investigated neutral and charged surfactants in NE for transdermal delivery of fludrocortisone acetate and flumethasone pivalate. Neutral surfactants included sucrose laureate and polysorbate-80 as nonionic surfactants. The positively charged surfactants were represented by PS. The physicochemical properties of the NE were evaluated in terms of particle size and zeta potential measurements. These properties were found to depend on the type of nonionic surfactant and the concentration of PS. Furthermore the cationic PS was found to offer a permeation rate enhancement factor between 1.1 and 1.5 when compared to the control, for fludrocortisone acetate and flumethasone pivalate, respectively. The interaction of porcine skin with positively and negatively charged NE was also evaluated by DSC analysis. DSC thermograms showed a slight difference in the phase transition temperature assigned to the characteristic lipid transition. However, it was not possible to assign the effect to one of the components in the multicomponent system [76].

Klang *et al.* evaluated negatively and positively charged NE without the use of conventional synthetic surfactants for transdermal delivery of progesterone. Natural substances such as sucrose esters and CDs were additionally added as stabilizing agents. The optimized NE were investigated for their potential as DDS for progesterone. Particle size and zeta

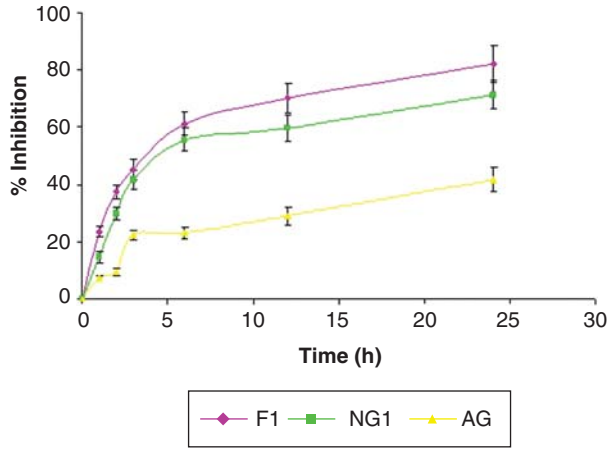

Figure 3. Comparative *in vivo* anti-inflammatory effects of aceclofenac from nanoemulsion (F1), nanoemulsion gel (NG1) and conventional aceclofenac gel (AG) in rats (n = 6).
Reproduced with permission of AAPS from [66].

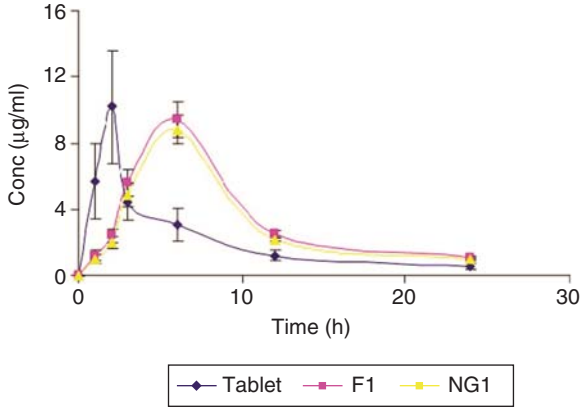

Figure 4. Pharmacokinetic profile of aceclofenac in rats (n = 6) following administration of nanoemulsion (F1), nanoemulsion gel (NG1) and marketed tablet formulation (Tablet).
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potential were also monitored for more than 1 year. The effect of the natural excipients sucrose stearate and CDs α, β and γ on *in vitro* skin permeation was evaluated. The CDs were found to induce fundamental changes in formulation structure as confirmed by cryo-TEM, thus leading to increased skin permeation profile of progesterone as compared to control [77].

Kim *et al.* developed a NE formulation of ketoprofen using benzyl alcohol/ethanol/Solutol/Smash® HS 15/water. Solutol from BASF is polyglycol monoethylene and diesters of hydroxyl stearate. These NE systems showed a high degree of stability because the droplet size did not change over a period of at least 3 months. The NE formulation containing 4% benzyl alcohol showed a higher permeation rate than what was observed with the 1 and 2% benzyl alcohol NE. Also the NE system containing 1% Solutol® HS 15 showed a permeation rate higher than was seen with 2% and 4% NE. All ketoprofen-loaded NE showed enhanced *in vitro* permeation rate through mouse skin as compared to the control [78].

Sakeena *et al.* evaluated NE system for *in vitro* skin permeation and skin irritation potential of ketoprofen using excised rat skin. *In vitro* skin permeation of ketoprofen NE through rat skin was performed in Franz diffusion cell and compared with marketed Fastum gel®. The limonene was also added as a permeation enhancer in NE system at the level of 1%, 2%, and 3% and its skin permeation profile was evaluated. Addition of limonene was found to increase the skin permeation of ketoprofen from NE with increasing concentrations of limonene. The results showed that NE containing 3% limonene produced similar and comparable skin permeation of ketoprofen with the marketed formulation. Skin irritation scores of optimal NE were compared with control and marketed Fastum® gel. These studies showed that optimal NE formulation was safe for transdermal delivery of ketoprofen [79].

Madhusudhan *et al.* studied *in vitro* skin permeation of nabumetone across rat skin from NE of soybean oil stabilized with a blend of lecithin and 1-*O*-alkylglycerol (C_{10}, C_{12}, C_{14}, or C_{16} chain length). The mean droplet size of the NE was found to be in the range of 214 – 280 nm. 1-*O*-Alkylglycerol stabilized NE showed significant enhancement in the *in vitro* skin permeation profile of nabumetone. This component-assisted permeation was attributed to the interaction of 1-*O*-alkylglycerol with the skin lipids. The stabilized NE showed highest flux in the first 4 h and did not exhibit any lag time. The other NE showed increase in lag time with increase in the chain length of 1-*O*-alkylglycerol. Overall, these results indicated that NE could be good vehicle for transdermal delivery of nabumetone [80].

Alves *et al.* developed and characterized semisolid topical formulations containing nimesulide-loaded nanospheres, nanocapsules or NE. The nanoprecipitation and spontaneous emulsification methods were used to prepare the nanosuspensions and the NE, respectively. The hydrodynamic diameters were found to be less than 300 nm for all the nanovehicles. Each nimesulide-loaded nanovehicle formulation was loaded into Carbopol 940® gels, characterized, and evaluated. The content of nimesulide and the pH values for all the gels were found to be constant during the storage of 120 days. The rheograms of all formulations were found to exhibit a non-Newtonian behavior presenting pseudoplastic characteristics and shear thinning [81]. In another significant study, Alves *et al.* investigated the *ex vivo* skin penetration of nimesulide from these nanovehicles. Nanovehicles were incorporated in the hydrophilic gels and their ability of delivering the drug into the human skin was investigated using stripping technique and Franz-type diffusion cells. The amount of nimesulide penetrated into the stratum corneum from the gel containing nanocapsules (GNM-NC) and the gel containing nanospheres (GNM-NS) was found to be similar. On the other hand, for the gel containing NE (GNM-NE), the nimesulide was not quantified in SC, but it has been directly

penetrating the dermis. The *ex vivo* penetration of the nimesulide from the GNM-NC was larger in the deeper skin layers than from the GNM-NS or GNM-NE. The gels containing nanovehicles were able to penetrate the drug in the viable layers of the skin as compared to a nonparticulated nimesulide-loaded formulation at the same concentration of nimesulide [82].

Kumar *et al.* investigated a novel o/w NE system for transdermal delivery of antihypertensive drug amlodipine. Various NE formulations were prepared by spontaneous emulsification method using oleic acid as oil phase, Tween 20 as surfactant, and Transcutol P as cosurfactant. The effects of content of oleic acid and mass ratio of surfactant/cosurfactant on skin permeation of amlodipine were investigated through excised rat skin in a Franz diffusion cell. Highest diffusion rate and permeability coefficient were obtained at concentrations of oil and surfactant/cosurfactant. The optimized NE formulation of amlodipine containing 2% oleic acid, 20% Tween 20, 10% Transcutol-P, and 68% water showed highest J_{ss} of 49.681 ± 1.98 μg/cm^2/h and K_p of 0.497 ± 0.056 cm/h. These results suggested that NE are potential vehicles for improved transdermal delivery of amlodipine [83].

Dixit *et al.* developed and evaluated the potential of NE for increasing the solubility and the *in vitro* transdermal delivery of carvedilol. Transdermal permeation of carvedilol through rat abdominal skin was determined with Keshary–Chien diffusion cell. Significant increase ($P < 0.05$) in the Jss and Kp was observed in NE formulations as compared to control or drug-loaded neat components. The highest value of these permeability parameters was obtained in optimized formulation B3, which consisted of 0.5% carvedilol, 6% oleic acid:IPM (1:1), 22.5% Tween 80, 22.5% Transcutol P, and 49% distilled water and in which the solubility of the drug was 4500-fold higher. The optimized NE was characterized for pH, conductivity, viscosity, droplet size, droplet shape, and refractive index. Thermodynamic studies showed that there had been a significant decrease of 88% in activation energy when the drug was incorporated in NE [84].

Akther *et al.* investigated the NE system for enhanced *in vitro* as well as *in vivo* percutaneous penetration of domperidone. Nine NE formulations were selected, characterized, and their *ex vivo* permeation studies using rat skin were performed. The NE formulations had small droplet size (< 90 nm), uniform size distribution (PI < 0.2), and low viscosity. The results demonstrated that the droplet size and viscosity of NE decreased following decrease in the concentration of Polysorbate-20, whereas transdermal flux was increased. The optimized formulation NE-B1, which contained oleic acid (4%), Polysorbate 20 (10%), Transcutol-P (20%), and water (64%) showed significant increase ($P < 0.01$) in the transdermal flux (169.32 ± 8.33 μg/cm^2/h). The *in vivo* studies revealed a 3.5-fold increase in relative bioavailability through transdermal application of optimized NE formulation compared to oral drug suspension. Moreover, the effective drug plasma concentration was maintained for 16 h after the transdermal application indicated that the developed NE systems could be a promising vehicle for the transdermal delivery of domperidone for prolonged period [85].

Azeem *et al.* investigated NE as nanodrug vehicles for the percutaneous delivery of ropinirole. They found enhanced transdermal delivery of antiparkinsonian drug ropinirole through NE as compared to NE gel [86]. In another significant research, Azeem *et al.* precisely focused on pharmacokinetic, biochemical, and mechanistic assessment of transdermal NE gel (NEG) of ropinirole in rats induced with Parkinson lesioned brain by 6-hydroxydopamine (6-OHDA). Mechanistic studies (DSC and FT-IR) showed that NEG affects the normal lipid packing of stratum corneum to enhance the drug permeation. Pharmacokinetic investigations revealed a greater and more extended release of ropinirole from NEG as compared to a conventional gel and oral marketed tablet (Ropitor®). Furthermore, biochemical investigations showed better activity from ropinirole NE as compared to marketed tablet. Finally, they concluded that NEG of ropinirole could be suitable for clinical treatment of Parkinson's disease [87].

Zhu *et al.* prepared lidocaine NE and investigated the transdermal delivery ability *in vitro*. The diameter and distribution range were detected by particle size analysis instrument, and the morphology of the NE was observed by TEM. The permeation flux of lidocaine was determined *in vitro* using the modified Franz diffusion cell combined with HPLC, and the cumulative transdermal absorption amount and the apparent skin transdermal velocity were compared among NE, gel, and tincture containing 5% lidocaine. The permeation mode of lidocaine NE was also analyzed. The average droplet size of lidocaine NE was 29.8 nm, and 98% of the drop sizes ranged from 15.1 to 45.5 nm and 2% from 77.9 to 261.3 nm. The NE droplet showed a spherical morphology in a polydisperse system. The K_p value of the NE (3.07 cm/h) was significantly higher than that of gel (1.27 cm/h) and tincture (0.97 cm/h), and the permeation rate of the NE was 69.82 μg/cm^2/h, which fitted the zero-order release dynamic procedure. These results indicated that the NE system with high permeation rate may provide a new promising means for local anesthesia [88].

Izquierdo *et al.* investigated the influence of emulsion droplet size on the skin penetration of a model drug tetracaine. *In vitro* dermal and transdermal delivery of tetracaine from six emulsions (three macro-emulsions and three NE) were evaluated. The results from emulsions were different only in droplet size and did not provide statistically significant evidence for the anticipated increase in transdermal or dermal delivery after 24 h application. The same results were obtained when the surfactant concentration in the aqueous phase was kept constant which indicated that there is no influence of emulsion droplet size on the skin penetration of tetracaine. These results indicated that this is in contrast to what has been reported in various publications that claimed penetration to increase with reducing droplet size [89].

Xiaoliang *et al.* investigated and analyzed the percutaneous permeation efficiency of NE preparation containing fluorescence tracer agent Bentex® OB. Prepared NE were

characterized in terms of droplet diameter, distribution range, and appearance. The tincture, IPM, and Labrasol/Plurol Oleique solutions containing OB were taken as the controls. The skin permeation path of each preparation in the skin was visualized and the permeation efficiency difference was analyzed. The mean droplet size of OB-NE was found to be 65.4 nm and the droplet size distribution ranged between 39.9 and 102.7 nm. The appearance of OB-NE droplet was found to be spherical in shape and the fluorescent intensity value in the superficial dermal layers of NE was higher than the tincture, IPM, and Labrasol/Plurol Oleique control groups. These results indicated that the NE preparation has singular skin permeation efficiency and could be a new promising cutaneous penetration preparation [90].

Puglia *et al.* evaluated *in vitro* drug release and anti-inflammatory effects of glycyrrhetic acid (GA) through the human skin from the NE system. GA-loaded NE (GAN) was prepared using PIT method and was characterized in terms of mean droplet size, morphology, and stability. The GA release from NE was evaluated *in vitro* to determine its percutaneous absorption through excised human skin. Prepared NE showed a mean droplet diameter of 210 nm that drastically changed during storage of 5 weeks at room temperature. *In vitro* evidence indicated that the NE system significantly increased the transdermal permeability of GA as compared to a control emulsion containing the same amount of GA. *In vivo* studies indicated that the NE system significantly increased the anti-inflammatory effects of GA as compared to the control. These results indicated that NE could be a good vehicle for transdermal delivery of GA [91].

Zheng *et al.* developed and evaluated NE system for transdermal delivery of granisetron hydrochloride. Pseudo-ternary phase diagram was constructed to identify the NE zone and concentration range of components of NE composed of IPM as an oil phase, Tween-85 as surfactant, ethanol as cosurfactant, and water as aqueous phase. The effects of the IPM as an oil phase and *n*-methyl pyrrolidone (NMP) as skin permeation enhancer on skin permeation of granisetron hydrochloride NE were also evaluated *in vitro* using rat skin. The optimum formulation which composed of 2.5% granisetron hydrochloride, 4% IPM, 40% Tween-85/ethanol (1:1) and 10% NMP showed that the skin permeation rate was the highest (85.39 ± 2.90 μg/cm^2/h) and enhancement factor was 4.1-fold for transdermal NE as compared with the control group. The cumulative amount of drug permeation was also highest (891.8 ± 2.86 μg/cm^2) with the shortest lag time (0.11 ± 0.02 h) and was stable for at least 12 months. From these studies, it was concluded that the NE system could be a promising vehicle for the transdermal delivery of granisetron hydrochloride, which could be as effective as oral or intravenous dosage forms and avoid some difficulties associated with these dosage forms [92].

Kuo *et al.* investigated whether a subcutaneous injection and/or transdermal application of a NE preparation of ASF would reduce tumor growth rate in a neuroblastoma xenograph mouse model. They found that subcutaneous and/or transdermal application of an ASF NE preparation was effective in reducing tumor growth rate in this neuroblastoma mouse model [93].

Kotyla *et al.* compared the transdermal application of a nano-sized emulsion versus a micron-sized emulsion preparation of delta tocopherol in hamsters. Each emulsion preparation of delta tocopherol contained canola oil as oil, Polysorbate-80 as surfactant, and deionized water as the aqueous phase. Both emulsions were formulated into a cream and applied to the shaved dorsal area of animals. The particle size of the micron-sized emulsion was found to be 2788 nm compared to the nano-sized emulsion (65 nm). Two hours post-application, hamsters that were applied the nano-sized emulsion were found to have a 36-fold significant increase in plasma delta tocopherol level, whereas hamsters that were applied as micron-sized emulsion were found to have only a ninefold significant increase, compared to the baseline respectively. At 3 h post-application, plasma delta tocopherol were found to have a 68-fold for hamsters applied as nano-sized emulsion, whereas only an 11-fold significant increase was observed in hamsters applied to micron-sized emulsion compared to baseline, respectively. This investigation suggested that nano-sized emulsions significantly increase the bioavailability of transdermally applied delta tocopherol as compared to the micron-sized emulsion [94].

Yilmaz and Borchart evaluated the effect of positively charged o/w NE containing ceramide 3B and naturally found SC lipids (PNSC) such as ceramide 3, cholesterol, and palmitic acid on skin hydration, elasticity, and erythema. Creams of PNSC were compared to NE creams. The formulations (NE, PNSC, and NNSC) were prepared by HPH. After adding Carbopol 940 as thickener, particle size and stability of the creams were not significantly changed as compared to the NE. The studies were carried out on three groups, each with 14 healthy female test subjects between 25 and 50 years of age, using Corneometer 825, Cutometer SEM 575, and Mexameter 18 for measurements of skin hydration, elasticity, and erythema of the skin, respectively. All formulations increased skin hydration and elasticity. There was no significant difference between PNSC and Physiogel. However, PNSC was significantly more effective in increasing skin hydration and elasticity than NE and NNSC indicating that PS induced positive charge. It was also concluded that SC lipids and ceramide 3B are crucial for the enhanced effect on skin hydration and viscoelasticity [95].

Calderilla-Fajardo *et al.* investigated the influence of sucrose laureate and sucrose oleate on the *in vivo* percutaneous penetration of octyl methoxycinnamate (OMC) formulated in colloidal suspensions such as NE, nanocapsules, and conventional o/w emulsions. The results of these studies showed that NE prepared with sucrose laureate exhibited the highest penetration in the stratum corneum compared to the other formulations. A twofold increase in OMC skin deposition was observed in case of NE containing sucrose laureate as compared to the control. The obtained data suggested that the total amount of OMC detected in the stratum corneum and the penetration

depth are strongly dependent upon the formulation's nature, the particle size, and the type of enhancer [96].

7. Future studies

NEs have been investigated as potential vehicles for enhancement of dermal and transdermal delivery of various hydrophobic compounds. These nanovehicles have shown promise in various models ranging from *in vitro* to *in vivo*. Although a number of research articles have been presented within the scope of this review, research is still limited and must be expanded to elucidate fully the potential for application of NE-based drug delivery systems for the design of more efficient dermal and transdermal formulations. The majority of work done to date in the field of NE-based dermal and transdermal drug delivery systems involves the use of rats or mice as animal model for *in vitro/ex vivo* permeation studies as well as for *in vivo* therapeutic/pharmacokinetic studies. Further studies regarding the safety, efficacy, and pharmacokinetic profiles of the hydrophobic compounds using NE must be performed on human beings and other animal models such as rabbits and beagle dogs, etc. More studies regarding the short- and long-term toxicity of NE both on animal models as well on human beings are also required. Fundamental research on the role of surfactants in NE production is also required to optimize emulsifier systems properly. NE can be explored for targeted delivery of genes, vaccines, and anticancer agents which can hold significant promise in the area of nanobiotechnology and oncology for the treatment of various tumors and other chronic diseases. Finally, scale-up considerations for the manufacture of dermal/transdermal NE are another issue that needs to be addressed in order to make the NE approach feasible.

8. Conclusions

NE have been investigated extensively for the dermal and transdermal delivery of anti-inflammatory drugs, anticancer drugs, antihypertensive drugs, central nervous system (CNS) drugs, anti-infective drugs, natural antioxidants, and phytotherapeutics. For enhancement of dermal and transdermal drug delivery of hydrophobic compounds, one must modify their physicochemical properties such as intrinsic solubility, diffusion coefficients, steady-state flux, and partition coefficients through the skin. Many natural as well as synthetic chemical enhancers have been investigated to modify these physicochemical properties to enhance dermal and transdermal delivery of certain hydrophobic compounds. Many of these chemical enhancers have been investigated to cause skin irritation or skin toxicity upon long-term use of dermal or transdermal drug delivery system. Many *in vitro* and *in vivo* (preclinical) studies on several hydrophobic compounds have proved the feasibility of the NE as potential vehicles for dermal and transdermal applications. Nevertheless, clinical investigations have rarely been performed on dermal and transdermal NE systems and more *in vivo* studies are also required to support the *in vitro* data.

9. Expert opinion

NEs have been formulated using a variety of pharmaceutically acceptable oils, surfactants, cosurfactants, and aqueous phases. Their principal advantages as vehicles for dermal and transdermal drug delivery system are higher solubilization capacity, ease of preparation, and thermodynamic stability. In many cases of dermal and transdermal NE, skin irritation or skin toxicity issues in human beings have not been considered which are a very important part of dermal and transdermal drug delivery system. Local and systemic toxicity studies should also be performed to evaluate therapeutic benefit/risk ratio. It should be noted that most of the studies conducted with dermal and transdermal NE of hydrophobic compounds are done *in vitro* or *in vivo* on animal models which shows greater penetration than human beings. The design of such experiments should be done carefully because human membranes behave in a different manner from membranes taken from animal species. In the last decade, much attention has been given to exploring new types of NE vehicles for dermal and transdermal delivery of many hydrophobic compounds. More advanced characterizing techniques such as NMR, DSC, FTIR, PCS zetasizer, and TEM, etc. are becoming available; therefore, it becomes very easier to characterize the correlation between the nature of the nanodroplets and the bioavailability of the embedded drug. Some NE-based formulations have recently been approved for clinical use and several NE-based products are still in the pipelines of various phases of clinical trials. All these marketed formulations have either been approved for oral drug delivery or topical drug delivery. Still there is no NE formulation for transdermal applications in the market. The main reasons for low industrial applications of transdermal NE are low permeability of most of the drugs through human skin, scale-up problems of NE, limitations of safe materials used for preparation of NE, lack of interaction studies between NE and human skin, and lack of preclinical/clinical data on human skin. Therefore, there is a need to investigate the interactions of NE with human skin properly. More irritation/toxicity studies should also be carried out on human skin in relation to concentration of surfactant/cosurfactant. Finally, the synergistic/antagonistic effects of these nanovehicles with other physical enhancement methods such as iontophoresis and sonophoresis, etc. should be investigated. Therefore, this area of research would be very useful for formulation scientists in order to develop some NE formulations for clinical applications.

Declaration of interest

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